

## PATENT COOPERATION TREATY

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## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 31 MAR 2005

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Applicant's or agent's file reference BW330R/RVP/rmp		<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA416)	
International application No. PCT/IB 03/05092	International filing date (day/month/year) 12.11.2003 ✓	Priority date (day/month/year) 13.11.2002 ✓	
International Patent Classification (IPC) or both national classification and IPC C12N15/82			
Applicant PLANTECHNO SRL et al. ✓			
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 5 sheets, including this cover sheet. ✓</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 3 sheets. ✓</p>			
<p>3. This report contains indications relating to the following items:</p> <p>I <input checked="" type="checkbox"/> Basis of the opinion</p> <p>II <input type="checkbox"/> Priority</p> <p>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p>IV <input checked="" type="checkbox"/> Lack of unity of invention</p> <p>V <input checked="" type="checkbox"/> Reasoned statement under Rule 68.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</p> <p>VI <input type="checkbox"/> Certain documents cited</p> <p>VII <input type="checkbox"/> Certain defects in the international application</p> <p>VIII <input type="checkbox"/> Certain observations on the international application</p>			
Date of submission of the demand 07.06.2004 ✓		Date of completion of this report 31.03.2005	
Name and mailing address of the international preliminary examining authority:  European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel.: +31 70 340 - 2040 Tlx: 31 651 epo nl Fax: +31 70 340 - 3016		Authorized Officer Bucka, A Telephone No. +31 70 340-2279 	

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IB 03/05092

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17):*

**Description, Pages**

1-5, 7-11, 13-23

as originally filed

6, 12

received on 24.06.2004 with letter of 23.06.2004

**Claims, Numbers**

1-12

received on 25.02.2005 with letter of 23.02.2005

**Drawings, Sheets**

1/26-26/26

as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☒ furnished subsequently to this Authority in written form.  
☒ furnished subsequently to this Authority in computer readable form.  
☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☒ the claims, Nos.: 13-21  
☐ the drawings, sheets:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/IB 03/05092

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).  
*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees, the applicant has:
- ☒ restricted the claims.
  - ☐ paid additional fees.
  - ☐ paid additional fees under protest.
  - ☐ neither restricted nor paid additional fees.
2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is
- ☒ complied with.
  - ☐ not complied with for the following reasons:
4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:
- ☒ all parts.
  - ☐ the parts relating to claims Nos. .

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims	1-12
	No: Claims	
Inventive step (IS)	Yes: Claims	1-12
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-12
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IB 03/05092

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

- 1 Reference is made to the following documents:
  - D1: WO 99/03985 A (US AGRICULTURE) 28 January 1999
  - D2: KRISHNAMURTHY K ET AL: "Expression of wheat puroindoline genes in transgenic rice enhances grain softness" NATURE BIOTECHNOLOGY, NATURE PUBLISHING, US, vol. 19, no. 2, February 2001, pages 162-166, XP002229773
  - D3: WO 00/08161 A (TAKAIWA FUMIO ; UTSUMI SHIGERU (JP); BIO ORIENTED TECHNOLOGY RESEAR (J) 17 February 2000
  - D4: WATANABE M ET AL: "CONTROLLED ENZYMATIC TREATMENT OF WHEAT PROTEINS FOR PRODUCTION OF HYPOALLERGENIC FLOUR" BIOSCIENCE BIOTECHNOLOGY BIOCHEMISTRY, vol. 58, no. 2, 1 February 1994, pages 388-390, XP000435777
  - D5: EP-A-1 190 624 (ENZYMES GMBH AB) 27 March 2002
  - D6: TSENG C -S ET AL: "Physicochemical properties of wheat flour dough modified by microbial transglutaminase" JOURNAL OF FOOD SCIENCE, vol. 67, no. 2, March 2002, pages 750-755, XP008037713
- 2 The subject matter of present **claims 1 to 12** meets the requirements of Article 33(2) PCT.
- 3 Document D1, which is considered to represent the most relevant prior art, discloses the modification of flour by the introduction into a plant of an additional gene encoding a storage protein. This approach is exemplified by the transformation of wheat, oat and barley with constructs encoding storage proteins of wheat. D2 and D3 are documents, which describe the modification of the characteristics of rice grains, e. g. by the introduction of puroindoline and soybean glycinin genes, respectively.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/IB 03/05092

The subject matter of **claim 1** differs from the subject matter disclosed in document D1 in that it relates to flour obtained from plants transformed with constructs encoding wheat storage proteins (glutenins) **and** the enzyme transglutaminase. The problem to be solved by the present application is considered to reside in the provision of further modified cereal or *Leguminosa* plants, which supply flour with the ability to rise.

The solution proposed by the present application consists of the transformation of cereals, with the exception of wheat, and *Leguminosa*, respectively, with constructs encoding wheat storage proteins and a transglutaminase. The resulting flour has the ability to rise and is expected to show a low allergenicity.

Plants modified by the introduction of wheat storage proteins have been described. Document D1 not only describes this approach for the alteration of oat and barley, respectively, but also suggests the transformation of different plants, *inter alia* of rice and maize (D1, pages 18, 20).

Transglutaminase, on the other hand, has been used to modify the properties of flour in the state of the art (cf. D4, D5, D6). Transglutaminase has also been shown to reduce the allergenicity of wheat proteins (cf. D4, tables I to III).

However, none of the cited documents suggests the introduction of genes coding for wheat storage proteins **and** transglutaminase into seed plants in order to modify the properties of flour obtained therefrom.

For these reasons, the subject matter of **claims 1 to 12** of the present application meets the requirements of Article 33(3) PCT.

CLAIMS

(48)

1. A non allergenic, rising food flour, derived from the seed of a plant expressing in said seed a gene coding for the transglutaminase enzyme and one or more gene coding for wheat storage proteins, wherein said wheat storage proteins comprises a preserved C-terminal motif LKVAKAQQLAAQLPAMCR and are selected from the group consisting of 1Bx7, 1By9, 1Dx5, 1Dy10, 1Ax2, 1Bx17, 1Ax1, 1Dy12, 1Dy10 and HMW2 and are optionally modified with techniques of site directed mutagenesis in order to eliminate allergenic aminoacid sequences for food allergies to gluten, and wherein said plant is a cereal or a leguminosa provided that said plant is not wheat.

2. The food flour of claim 1 wherein the sequences to be modified are selected from the group consisting of PFPQPQLPY, PQPQLPY, PYPQPQLPY, LQLQFFPQPQLPY, QQGYPTSPQSG, QQGYPTS, PFSQQQQQ, QSEQSQPFQPQ and QXPQQPQQF.

3. The food flour of claim 2 wherein the site directed mutagenesis is directed to aminoacid in position 6 for PFPQPQLPY, aminoacid in position 4 for PQPQLPY, aminoacid in position 6 for PYPQPQLPY, aminoacid in position 10 for LQLQFFPQPQLPY, aminoacids in position 5 and 8 for QQGYPTSPQSG, aminoacids in position 5 and 8 for QQGYPTS, aminoacids in positions 4,5 and 7 for PFSQQQQQ, aminoacids in positions 4 and 6 for QSEQSQPFQPQ, aminoacid in position 4 for QXPQQPQQF.

4. The food flour of anyone of claims 1 to 3 wherein the cereal plant is rice, soybean or corn.

5. A transgenic plant expressing in seed a gene coding for the transglutaminase enzyme and one or more gene coding for wheat storage proteins, wherein said wheat storage proteins comprises a preserved C-terminal motif LKVAKAQQLAAQLPAMCR and are selected from the group consisting of 1Bx7, 1By9, 1Dx5, 1Dy10, 1Ax2, 1Bx17, 1Ax1, 1Dy12, 1Dy10 and HMW2 and are optionally modified with techniques of site directed mutagenesis in order to

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eliminate allergenic aminoacid sequences for food allergies to gluten, and wherein said plant is a cereal or a leguminosa provided that said plant is not wheat.

5 6. The plant of Claim 5 wherein the sequences to be modified are selected from the group consisting of PFPQQLPY, PQQQLPY, PYPQQLPY, LQLQFFPQQLPY, QQGYPTSPQQSG, QQGYPTS, PFSQQQQQ, QSEQSQPFQPFQ and QXPQQPQQF.

10 7. The plant of Claim 6 wherein the site directed mutagenesis is directed to aminoacid in position 6 for PFPQQLPY, aminoacid in position 4 for PQQQLPY, aminoacid in position 6 for PYPQQLPY, aminoacid in position 10 for LQLQFFPQQLPY, aminoacids in position 5 and 8 for QQGYPTSPQQSG, aminoacids in position 5 and 8  
15 for QQGYPTS, aminoacids in positions 4,5 and 7 for PFSQQQQQ, aminoacids in positions 4 and 6 for QSEQSQPFQPFQ, aminoacid in position 4 for QXPQQPQQF.

8. The plant of anyone of claims 5 to 7, wherein said plant is rice, soybean or corn.

20 9. A seed produced by the plant of anyone of claims 5 to 8 wherein said seed expresses the transglutaminase enzyme and one or more gene coding for wheat storage proteins, wherein said wheat storage proteins comprises a preserved C-terminal motif of LKVAQAQQLAAQLPAMCR and are  
25 selected from the group consisting of 1Bx7, 1By9, 1Dx5, 1Dy10, 1Ax2, 1Bx17, 1Ax1, 1Dy12, 1Dy10 and HMW2 and are optionally modified with techniques of site directed mutagenesis in order to eliminate allergenic aminoacid sequences for food allergies to gluten, and wherein said  
30 plant is a cereal or a leguminosa provided that said plant is not wheat.

10. A process for the production of flours from the seeds of Claim 9, comprising the step of milling said seeds.

35 11. A process for producing a baked product comprising the steps of admixing the flour as defined in anyone of claims 1 to 4 with a suitable amount of yeast,

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26

allowing said flour to raise and baking the obtained dough.

12. A baked product obtainable by the process of claim 11.